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A genetic approach to the identification of plant genes involved in viral movement

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13. ABSTRACT (Maximum 200 words)

Plant viruses encode a movement protein that is required for movement and systemic infection of host plants. We propose a genetic approach to identifying host proteins involved in plant viral movement. We are using as a model red clover necrotic mosaic virus (RCNMV) which infects, but does not kill *Arabidopsis*.. We plan to create two transgenic plant lines of *Arabidopsis*, one that expresses the firefly luciferase (Luc) gene, and one that expresses the *codA* (cytosine deaminase, CD) gene. Both genes will be under the transcriptional control of the RCNMV subgenomic promoter. We will identify transgenic plant lines that express Luc or CD upon infection with RCNMV. Phenotypically these lines will either be luminescent (Luc) or sensitive to 5-fluorouracil (5FU) (CD) when infected with RCNMV. A library of EMS mutants will be generated for Luc- and CD-expressing plant lines. Plantlets from the mutagenized Luc-expressing lines will be infected with RCNMV and screened for the loss of luminescence. Plantlets from the mutagenized CD-expressing lines will be infected with RCNMV and selected for resistance to 5FU. Surviving mutants will be examined for the presence of RCNMV in the inoculated and upper leaves. Mutants that are unable to support RCNMV infection will be selfed and their progeny tested as described above. The mutated gene in each of these lines will then be cloned and sequenced.

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REPORT DOCUMENTATION PAGE (SF298)
(Continuation Sheet)

A Genetic Approach to the Identification of Plant Genes Involved in Viral Movement

The mechanism(s) that plant viruses employ to move from infected cells into neighboring uninfected cells is poorly understood. Plant viruses, unlike animal viruses, must traverse a non-living cellulose wall surrounding each plant cell to move from one cell to the next, making receptor-mediated endocytosis and cellular fusion, as observed in animal virus infections, impossible. Most plant viruses encode one or more movement proteins that are required for cell to cell movement and systemic infection of host plants (Carrington et al., 1996). Several of these movement proteins have been extensively characterized and this has enabled certain functions to be associated with viral movement such as nucleic acid binding, enlargement of the plasmodesmatal channels between cells, and movement of nucleic acids from cell to cell (Lucas and Gilbertson, 1994). However, this information has shed little light on how movement proteins facilitate the spread of virus during infection. Specifically, several important questions remain: How does the movement protein modify plasmodesmata? By what means does it transfer the viral genome through the plasmodesmata? Do plant viruses usurp any host functions to achieve viral movement? In the short term we aim to develop a better understanding of how plant viruses cause disease and interact with host intercellular macromolecular trafficking infrastructure. In the long term, our goals are to use this information to develop novel control strategies for viral disease in general and to manipulate macromolecular traffic between plant cells.

To this end, we have undertaken a genetic approach to identifying host proteins involved in plant viral movement, both cell to cell and long distance. This genetic approach complements biochemical and molecular experiments also underway in our laboratory. We are using as a model the movement protein (MP) of the well-characterized red clover necrotic mosaic virus (RCNMV), a 32 nm icosahedral, single-stranded, positive sense RNA virus. RCNMV does not infect *Arabidopsis thaliana*, but its MP can facilitate the movement of a reporter virus that does infect Arabidopsis.

Originally, we proposed a screen of mutagenized Arabidopsis for mutant plants that were resistant to RCNMV infection based on the transgenic plant lines expressing reporter genes under the transcriptional control of the RCNMV subgenomic promoter. However, recent work indicates that the RCNMV subgenomic promoter is only effective in its native viral context. Therefore, a modification of our original proposal is being employed. This modification has the additional advantage of not requiring the generation of transgenic plants, thereby potentially accelerating our search for plant mutants affecting RCNMV infection. The modifications make adjustments in two aspects of the originally proposed work, the virus delivery system and the reporter system. Due to its potential for efficient delivery of high virus titers to the host plant (Callaway et al., 1996), an agroinoculation system was developed. Since last year's report, concerns about potential problems with agroinoculation of this RNA virus have been allayed. We have demonstrated that both RCNMV and our reporter virus (turnip vein-clearing tobamovirus or TVCV) are efficiently delivered to host plants.

The second alteration is the choice of reporter. We have considered that in our susceptibility screen it may be that suboptimal interactions of mutant viral MP with supressor-mutant plant factors, will result in low viral titers. In order to detect even low amounts of virus in whole, living suppressor plants in a background of uninfected plants, a highly amplified vital reporter system is desirable.

Therefore, we are developing a movement-deficient form of an Arabidopsis-adapted virus expressing the green fluorescent protein (GFP) (Chiu et al., 1996) which we call a "reporter virus".

In the last year, we screened several viruses for the following essential features. The wild-type progenitor of the reporter virus should, of course, infect Arabidopsis. The functions of its gene products should be relatively well defined. Its genome should be flexible enough to allow the necessary molecular manipulations to a) make it dependent on RCNMV's (or another virus') MP for movement and b) insert a reporter gene (e.g. GFP) without disruption of native viral gene functions. Expression of the reporter should be stable enough to permit evaluation of viral infections throughout the entire life of an Arabidopsis plant. The movement of a MP-minus version of this reporter virus should be complemented in *trans* or in *cis* by the virus of interest (e.g. RCNMV). Turnip vein-clearing tobamovirus (TVCV) (Lartey et al., 1993) is the leading candidate for our reporter virus. In the last year, we have shown that it satisfies all of these criteria, except one. We have not yet tested a completed reporter virus construct designed to systemically infect Arabidopsis while stably expressing GFP to high levels. This construct and other reporter designs towards the same goal are being currently tested and are the only significant remaining barrier to our planned genetic screens for host factors involved in viral movement.

In summary, the overall scheme for isolation of plant mutants influencing the infection process of RCNMV in Arabidopsis involves two sets of screens of mutagenized Arabidopsis plants, one screen for plants resistant to infection by reporter virus bearing wild-type RCNMV MP (RV^{GFP}/ $wtMP^{RCNMV}$) and the other screen to look for plant suppressor mutants (Callaway 1998) that allow infection of reporter virus bearing movement-impaired RCNMV MPs available in the laboratory and described previously (Giesman-Cookmeyer and Lommel, 1993; Wang et al., 1998). In the first screen, M_2 mutagenized Arabidopsis plats will be inoculated with RV^{GFP}/ $wtMP^{RCNMV}$ and plants that fail to fluoresce under 488 nm excitation will be further analyzed as candidate mutant plants resistant to movement of the RCNMV chimera. In the second screen, M_2 mutagenized plants will be inoculated with RV^{GFP}/ mutant-MP^{RCNMV}. In this case, plants that fluoresce upon excitation with 488 nm light will be identified as candidate host mutants affecting RCNMV movement.

This past year we have expanded the project to engineer RCNMV to be able to move in Arabidopsis. To this end we have constructed a chimeric virus that requires RCNMV's MP to move, but otherwise is an Arabidopsis-infecting virus. The virus we are using is tobacco rattle tobravirus (TRV). TRV-based chimeras were constructed with no MP (negative movement control) and with the RCNMV-MP and inoculated onto *N. benthamiana*. The same RNA2 in all cases was used and it was the source of red-shifted GFP. Infection was difficult to see even in the wild-type TRV RNA1 positive control. Green fluorescent foci were seen under the microscope only in the wt TRV positive control. A few single-cell green fluorescent cells were observed with the TRV/ RCNMV MP chimera. Fluorescence faded rapidly in previous inoculations of the TRV wt control. Possibility that the eGFP used does not work as well in plants as the sGFP used in other (non-TRV-based) constructs. In a second approach we are modifying a known Arabidopsis-infecting virus so that it has suitable properties for the genetic screen. A mutation has been introduced in the MP of TVCV, a tobamovirus that infects Arabidopsis, based on analogous mutations of TMV's MP made in Roger Beachy's laboratory. This mutant virus infects *N. benthamiana* about as rapidly as wt TVCV, but without any of the usual necrosis (three independent inoculations). No visible symptoms are observed on Arabidopsis (one inoculation to date). RNA has been extracted and viral cDNA made from non-inoculated leaves of both hosts. A GFP-expressing version of this mutant virus is being made- I am screening colonies this week. We must know verify presence of introduced mutation in systemic tissue of infected *N. benthamiana* and inoculated Arabidopsis. Finish cloning GFP-version and inoculate plants.

As a backstop measure to these approaches we are also characterizing a crucifer-infecting sobemovirus: Turnip rosette (TRoV). To this end we are constructed an infectious clone of the virus. Previous T7 and 35S (Agro-delivered) "infectious" clones have not been successful. The presence of an additional nucleotide (compared to previous clones) at the 5' end of the viral genome has been observed in 2 independent 5'-RACE clones. T7 transcripts incorporating the new sequence

information have been made and inoculated onto Arabidopsis. Equivalent agroinoculation clone is being constructed.

Publications:

A. Callaway, D. Giesman-Cookmeyer, E. Gillock, T. L. Sit and S. A. Lommel. 2001. The Multifunctional Plant Virus Capsid Proteins. Annual. Review of Phytopathology. In press.

Callaway, A. and Lommel, S. A. 2001. A sobemovirus coat protein complements long-distance movement of a red clover necrotic mosaic dianthovirus lacking native coat protein. In preparation.